Proteomic Analysis of *Rickettsia prowazekii*

C. C. CHAO,[a] D. CHELIUS,[b] T. ZHANG,[b] E. MUTUMANJE,[a] AND W. M. CHING[a]

[a]*Rickettsial Diseases Department, Naval Medical Research Center, Silver Spring, Maryland, and Department of Preventive Medicine and Biometrics, Uniformed Services University of Health Sciences, Bethesda, Maryland, USA*

[b]*Thermo Electrons, San Jose, California, USA*

ABSTRACT: *Rickettsia prowazekii* is an obligate intracellular gram-negative bacterium. Comparative proteomics study of a virulent strain (Breinl) versus an avirulent strain (Madrid E) was performed using an integrated liquid chromatography and mass spectrometer. About 30% of predicted proteins were detected and identified. Among the detected proteins, more than 30 proteins were of unknown function in both strains. Although several proteins were detected in only one strain, the overall distribution of detected proteins in different COGs (clusters of orthologs groups) was very similar between the two strains. Functional analysis of differentially expressed proteins, either qualitatively or quantitatively, may lead to the discovery of pathogenesis-related factors.

KEYWORDS: *Rickettsia prowazekii*; proteomics; LC-MS-MS

INTRODUCTION

R. prowazekii is the causative agent of epidemic typhus. The natural infection is normally caused by rubbing of infected louse or its feces into a bite or skin abrasion and has caused significant health problems for military personnel during times of war and civil disturbance.[1,2] Infection can also occur by inhalation, permitting aerosolized *R. prowazekii* to be deployed as a biological weapon.[1] Consequently, *R. prowazekii* is listed as a select agent by the United States.[1,2]

The genomic DNA sequence of the avirulent strain of *R. prowazekii* (Madrid E) has been completed.[3] Differences in the genome of avirulent and virulent strains are evident as shown by Ge and colleagues[4] in that 24 genes in the avirulent Madrid E strain have significantly lower hybridization signals than in the virulent Breinl strain. These results suggest that some of these genes may be important for strain virulence. In this report, we analyzed the protein expression profiles of these two strains.

Address for correspondence: Chien-Chung Chao, Ph.D., RDD/IDD/NMRC, 503 Robert Grant Ave., RM 3N71, Silver Spring, MD 20910. Voice: 301-319-7313; fax: 301-319-7460.
chaoc@nmrc.navy.mil

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TABLE 1. Comparison of proteins detected in Madrid E and Breinl strains of *R. prowazekii*

Description	Madrid E	Breinl
Total detected proteins	251	250
Single peptide in all three runs	14	13
Multiple peptides in only one run	8	9
Multiple peptides in two or three runs	229	228
Hypothetical proteins detected	68	66

MATERIALS AND METHODS

Preparation of Rickettsia prowazekii

The preparation of *Rickettsia prowazekii* was carried out as described previously.[5]

Reduction, Alkylation, Digestion of Proteins, 2D-LC/MS/MS, and Data Analysis

The lysed rickettsia cells were reduced, alkylated, and digested with trypsin, and the digested sample (15 μg of total protein) was analyzed as described by Chao and colleagues.[5]

RESULTS AND DISCUSSIONS

The total number of proteins detected in Madrid E and Breinl strains were 251 and 250 (TABLE 1), respectively. This is equivalent to 30% of the total number of proteins based on ORFs. This limited efficacy may be due to the broad dynamic range of the expressed proteins. Among these detected proteins, 37 from Madrid E and 33 from Breinl were of unknown function. Most of the proteins were identified by multiple peptides or by one single peptide in all three runs. The distribution of detected proteins in different COGs was very similar between the two strains. Among the 24 genes considered as potential DNA vaccine candidates,[6] 12 of them were confirmed expressed (RP333, 344, 043, 775, 326, 833, 451, 586, 290, 291, 292, 293). Three of these 12 proteins (RP333, RP775, and RP586) were identified only in Madrid E and not in Breinl strain. Understanding the role of these three proteins is important to better comprehend the differences between the virulent and avirulent strains.

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[*Conflict of interest statement:* The authors of this research declare that they have no conflict of interest.]

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